Epigenetic Deregulation of DNA Repair and Its Potential for Therapy

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Abstract

Epigenetic silencing of essential components of DNA repair pathways is a common event in many tumor types, and comprise O6-methylguanine-DNA methyltransferase (MGMT), human mut L homolog 1 (hMLH1), Werner syndrome gene (WRN), breast cancer susceptibility gene 1 (BRCA1), and genes of the Fanconi anemia pathway. Most interestingly, some of these alterations become the Achilles heel of the affected tumors upon treatment with certain classes of anticancer agents. That is, patients whose tumors carry such defects can be stratified for respective therapy rendering some classic DNA damaging agents, such as alkylators or DNA crosslinking agents, into “targeted therapies.” Here we review some of the affected repair pathways that, when inactivated, sensitize the tumors to specific drugs and are thus exploitable for individualized therapy. (Clin Cancer Res 2009;15(16):5026–31)

Background

Epigenetic inactivation of genes by promoter methylation has been recognized as an important mechanism by which tumor suppressor genes are shut down during development of tumors. It represents one of the best studied mechanisms of aberrant epigenetic modification in cancer. It is an integrated part of the epigenetic regulatory system of gene expression involving changes in chromatin structure by post-translational modifications of histones and nucleosome remodeling. Hypermethylation of CpG islands in the promoter region leads to silencing either by direct inhibition of transcription factor binding, or by attracting methylated-DNA binding proteins, recruiting other transcriptional repressors such as histone acetylases (HDACs) and histone methyl transferases, resulting in transcriptionally inactive chromatin. DNA methylation at the 5 position of cytosine is mediated by DNA methyltransferases (DNMTs; reviewed in ref. 1).

Similar to other molecular aberrations, different tumor entities exhibit their characteristic profiles of genes frequently affected by promoter hypermethylation (2). In accordance with the Knudson 2-hit model of tumor suppression, methylation of one allele is often accompanied by inactivation of the second allele by deletion, mutation, or methylation (3). Genes encoding DNA repair proteins have been identified to become inactivated during development of several tumor types by mechanisms such as deletion, mutation, and most commonly promoter hypermethylation. Selection for aberrant DNA repair pathways in cancer development is in accordance with the concept that DNA damage signaling acts as a guardian against activated oncogenes and tumor progression (4). Thus, inactivation of such repair pathways represents important alterations in the development and malignant progression of these tumors. Genes encoding proteins involved in DNA repair predominantly silenced by promoter methylation in some tumor types comprise MGMT (O6-methylguanine-DNA methyltransferase), hMLH1 (human mut L homolog 1), WRN (Werner syndrome gene), and genes of the Fanconi anemia (FA) pathway such as FANCF, FANCC, or FANCL (Fanconi complementation group) and including BRCA1 (breast cancer susceptibility gene 1) (refs. 5–8). Most interestingly, some of the epigenetic alterations in these repair pathways sensitize the affected tumors to treatment with certain classes of anticancer agents (Fig. 1).

DNA Repair Pathways Affected by Epigenetic Silencing in Tumors

Epigenetically impaired repair by O6-methylguanine-DNA methyltransferase. MGMT has become the most prominent of the epigenetically inactivated DNA repair genes, because its promoter methylation has been associated with benefit from alkylating agent therapy in malignant glioma (9), which was subsequently confirmed in clinical trials for newly diagnosed glioblastoma (10–13), and now serves as stratification or inclusion criteria for patients entering clinical trials. Evidence for prediction of benefit from multidrug regimens comprising cyclophosphamide has been provided for B-cell lymphoma (14).

MGMT rapidly reverses alkylation (including methylation) at the O6 position of guanine by transferring the alkyl-group to the active site of the enzyme, constituted by a cysteine (15). The DNA repair protein gets irreversibly inactivated in this suicide reaction, in fact this process is saturable, in that an excess of O6-methylguanine in the DNA can deplete MGMT in the cells. Unlike other repair systems, MGMT acts alone to restore guanine, and without formation of single strand breaks.
Although, O6-alkyl-guanine is not the primary site of alkylation by alkylating chemotherapy agents, it seems to be the most cytotoxic. An inactivated MGMT gene allows accumulation of this lesion in the DNA, which subsequent to incorrect pairing with thymidine triggers mismatch repair (MMR) thereby inducing DNA damage signaling and eventually cell death (16, 17). In accordance with this mechanism MMR-deficient cells are highly resistant to alkylating agents, even in the absence of MGMT. Thus, MGMT rapidly repairs the most cytotoxic lesion of alkylating agents, O6-alkyl guanine, thus reverting the therapeutic effect of alkylating drugs. Consequently, epigenetic inactivation of the MGMT gene by promoter methylation renders MMR-proficient cells more sensitive to alkylating agents. In accordance, mice overexpressing MGMT are more resistant to carcinogenesis induced by alkylating agents, whereas respective knock-out mice are more sensitive (18).

Tumors most commonly affected by epigenetic inactivation of MGMT comprise glioblastoma (45%), other glioma subtypes (WHO grade I to III; 20 to <90%, subtype specific), colon (38%), lung (27%), and head and neck cancer (28%), and lymphoma (25%) (refs. 5, 11, 14, 19).

The clinical relevance of epigenetic silencing of the MGMT promoter for benefit from alkylating agent therapy was shown in a randomized trial for newly diagnosed glioblastoma. Patients whose tumors contained a methylated MGMT promoter had a clear survival benefit from the addition of the alkylating agent temozolomide to standard radiotherapy with a median survival of 23.4 months [95% confidence interval (CI), 18.6–32.8] as compared with 12.6 months (95% CI, 11.6–14.4.8) in patients with an unmethylated MGMT. In patients treated with initial radiotherapy only, no difference in progression-free survival could be shown, and a much smaller difference in overall survival (11, 12). Similarly, and in concordance with the postulated predictive value of MGMT methylation, retrospective analyses of glioblastoma patients treated with alkylating agents showed a beneficial effect in presence of MGMT methylation, whereas no association of MGMT status could be shown for patients treated with radiotherapy only (20, 21).

New mechanistic in vitro evidence for the direct involvement of MGMT in glioblastoma response to alkylating agent therapy has been provided by the recent The Cancer Genome Atlas (TCGA) report on human glioblastoma (22). The mutation analysis of 601 genes in 91 matched tumor and/or normal samples identified a hypermutator phenotype in the recurrent glioblastoma of a subset of patients treated with alkylating agents, which was confined to tumors with a methylated MGMT status in six of seven cases. Interestingly, the gene mutation pattern was different in patients whose glioblastoma carried a methylated MGMT promoter, compatible with the deficiency to repair alkylated guanine residues. Moreover, in the six treated and MGMT-methylated glioblastoma that were hypermutated, at least one of the MMR genes MLH1, MSH2, MSH6, or PMS2 was mutated as compared with only one of 84 nonhypermutated, nontreated glioblastoma, which may suggest escape from MGMT methylation-mediated sensitivity to the alkylating drug by selection for MMR deficiency.

**Epigenetic inactivation of BRCA-Fanconi anemia pathway of DNA repair.** The genomic instability syndrome FA is an autosomal recessive disorder characterized by congenital abnormalities, bone marrow failure, cellular sensitivity to DNA-cross linking agents, and a genetic cancer susceptibility syndrome with an increased risk for acute myeloid leukemia, squamous cell carcinoma of the head and neck, or gynecologic cancer. Recent studies have revealed that proteins affected in this disease by mutation constitute a novel DNA-damage response network that also involves the breast cancer proteins BRCA1 and BRCA2 (also known as FANCD1). Germline mutations in BRCA1 or 2 are associated with a breast and ovarian cancer syndrome (reviewed in ref. 23).

In sporadic tumors the BRCA-Fanconi pathway is rarely inactivated by mutations, but frequently silenced by aberrant promoter methylation of BRCA1 or FANCC and occasionally FANCC or FANCL. BRCA1 methylation has been identified in breast (11%–14%) and ovarian cancer (5%–31%), and infrequently in lung and cervical carcinomas. FANCF promoter methylation has been detected in ovarian (21%), breast (17%), head and neck cancers (15%), non-small cell lung cancer (14%), and cervical carcinomas (30%) (reviewed in ref. 8), whereas FANCC and FANCL are infrequently methylated in leukemia (24). In contrast to BRCA1 and FANCF, there is no evidence for epigenetic silencing of BRCA2 in breast cancer (25), and it is only rarely present in ovarian cancers (26). No systematic studies are available linking epigenetic inactivation of the BRCA-FA pathway with specific subtypes of breast cancer (27).

An intact BRCA-FA pathway is required to repair DNA cross-links, stalled DNA replication forks, and double strand breaks. Eight of the BRCA-FA pathway proteins are subunits of an E3 ligase (complex 1) that is required to mono-ubiquitinate FANCD2, a critical step for the function of the BRCA-FA pathway. Mono-ubiquitinated FANCD2 interacts with BRCA2-FANCD1 and other repair proteins to form DNA damage-inducible foci (complex 2). Consequently, loss of function of any essential FA pathway component is expected to impair the pathway and predict sensitivity to DNA crosslinking agents such as cisplatin, supported by experimental evidence (28). The cisplatin-sensitive human ovarian cancer cell line 2008 has a methylated FANCF promoter that upon prolonged exposure to cisplatin has been shown to become resistant, mediated by restoration of FANCF expression associated with demethylation of the FANCF gene. This in vitro observation suggests that selection for demethylation of this gene, restoring of the FA pathway, may account for acquired resistance to cisplatin treatment in a clinical setting (29).

Tumors with inactivation of the BRCA-FA network may also respond to inhibitors of alternative DNA repair pathways. BRCA1 and BRCA2 are essential for the repair of double strand breaks and stalled replication forks by homologous recombination (HR). Cells deficient for BRCA1 and BRCA2 have been shown to be exquisitely sensitive to poly(ADP-ribose) polymerase (PARP) inhibitors (30, 31). Repair of damaged bases or regions of single-strand breaks through base excision repair (BER) requires PARP, a DNA-binding zinc finger protein that catalyzes the transfer of ADP-ribose residues from NAD+ to itself and other proteins such as XRCC1, a key component thought to initiate BER upon ADP-ribosylation (32). PARP-deficient, and therefore BER-deficient mice, develop normally but have high levels of sister chromatid exchange (a feature of HR), suggesting that HR compensates for loss of PARP-dependent BER. Consequently PARP inhibitors are attractive drugs for BRCA1- and 2-deficient tumors. In addition, PARP inhibitors may also sensitize to alkylating agents such as temozolomide as suggested in recent trials, because BER is an important
pathway repairing some of the lesions caused by these agents (reviewed in refs. 33, 34).

**Epigenetic inactivation of WRN gene.** Loss of function mutations in WRN are the cause of an autosomal recessive disorder characterized by premature aging, genomic instability, and predisposition to cancer. The WRN gene has been reported to be frequently silenced by DNA methylation in cancers of epithelial and mesenchymal origin, most commonly in colorectal and non-small cell lung cancer (38%), gastric (25%) and prostate cancer (20%), chondro-sarcomas (33%), and non-Hodgkin lymphoma (23%; ref. 7).

The WRN gene is ubiquitously expressed and belongs to the RecQ helicase family of proteins with an intrinsic 3′–5′ exonuclease activity. WRN is a major component of the DNA repair and replication machinery, and has been shown to interact with important DNA repair proteins such as DNA topoisomerase I, BLM helicase, PARP-1, and RAD52. In line with the premature aging syndrome in absence of WRN, this helicase is involved in telomere maintenance, and plays an important role in replicative stress because of its function in repair, and remodeling and elongation of the replication fork (for review see ref. 35).

Agrelo and colleagues reported that colorectal cancer cell lines with WRN promoter methylation are sensitive to the topo-isomerase inhibitor camptothecin and to mitomycin C. Most interestingly, the authors showed that colorectal cancers exhibiting WRN methylation indeed responded significantly better to the topo-isomerase I inhibitor irinotecan with a median overall survival of 39.4 months versus 20.7 months (7). Sensitivity to topo-isomerase I inhibitors in WRN-negative cells has been attributed to the conversion of treatment-induced single strand breaks into double strand breaks at a high frequency in absence of WRN (36). Hypermethylation of WRN in colorectal tumors could thus be a useful predictor of clinical response to topo-isomerase I inhibitors.

**Inactivation of mismatch repair through epigenetic inactivation of hMLH1 is a factor in treatment resistance.** The MMR gene hMLH1 is an essential component of the DNA MMR pathway and is frequently mutated in hereditary nonpolyposis colon cancer (HNPCC) also known as Lynch syndrome. Defects in MMR-associated genes are common in many cancer types and result in a mutator phenotype associated with microsatellite instability (37). The MMR system recognizes base–base mismatches and insertion or deletion loops (IDLs) in double-stranded (ds) DNA, and degrades the region of the error-containing newly synthesized strand, allowing the polymerase to correctly re-synthesize the second strand according to the template sequence.

Activation of the MMR pathway may trigger DNA damage signaling, a process which induces cell cycle arrest and can lead to cell death in case of major DNA damages (for review see ref. 38). In cancer, hMLH1 is the most commonly altered component of this postreplicative DNA MMR system. It heterodimerizes with PMS2 to form MutL alpha, which gets recruited by MutS alpha (MSH2–MSH6) or MutS beta (MSH2–MSH3), bound to a dsDNA mismatch. Assembly of the MutL–MutS complex in presence of replication factor C (RFC) and proliferating cell nuclear antigen (PCNA) is sufficient to activate endonuclease activity of PMS2. It introduces single-strand breaks near the mismatch and thus generates new entry points for the exonuclease EXO1 to degrade the strand containing the mismatch.

hMLH1 is the most prominent target of epigenetic silencing in the MMR pathway in sporadic tumors, comprising ovarian, head and neck, breast, and colorectal cancer (6). However, hypermethylation of hMLH1 is often associated with other hypermethylated genes, which complicates mechanistic interpretation of associations with response to therapy in patients (39). Mechanistic investigations in vitro have shown that treatment of hMLH1-methylated colon cancer cell lines with the demethylating agent 5’aza-2’deoxycytidine (5-aza-dC) restores hMLH1 expression and subsequently renders the cells MMR proficient (6). In accordance, 5-aza-dC treatment-mediated re-expression of hMLH1 overcomes in vitro resistance to 5-fluorouracil (5-FU) in colorectal cancer cell lines (40).

A new, but rare, mechanism of epigenetic inactivation of MLH2 has been reported recently from families with Lynch syndrome, exhibiting inheritable somatic methylation of the MSH2 gene (41). This epigenetic inactivation by methylation is mediated by deletion of the last exons of the immediate upstream gene ACSTD1 in cis, and depends on the expression activity of the deletion mutant. Expression of the truncated ACSTD1, lacking a normal polyadenylation signal, results in transcriptional read-through into the MLH2 gene. In the affected patients, normal tissues positive for Ep-CAM (encoded by ACSTD1), were associated with a methylated MLH2 gene in contrast to Ep-CAM negative tissues.

### Clinical Translational Advances

**Stratifying patients according to epigenetically impaired repair by MGMT and MGMT modulation.** The clinical confirmation that glioblastoma patients indeed benefit from alkylating agent therapy dependent on the presumed nonfunctionality of DNA repair by MGMT, as determined by its promoter methylation status, has led to a paradigm change in the treatment of these patients. Whereas the association of MGMT methylation with benefit from alkylating agent therapy is prospectively assessed, attempts are taken to deplete the MGMT protein in the tumors of patients with an unmethylated MGMT gene using a dose dense schedule of the alkylating agent (13), or treatment with non-toxic inhibitors of the MGMT enzyme such as O6-benzylguanin, or derivatives (42, 43). New trials select patients according to the MGMT methylation status in order to test new drugs in combination with alkylating agent therapy expected to work best in patients with MGMT methylated tumors (NCT00689221); patients with MGMT unmethylated tumors, thus proficient for repair by MGMT, are enrolled for studies testing new strategies based on molecular mechanisms other than DNA alkylation (NCT00509821). Another avenue is taken in the development of a new generation of alkylating drugs, yielding O6-guanine adducts that are not susceptible to repair by MGMT. However, similar to strategies aiming at depleting MGMT in the tumor tissue using systemically delivered non toxic MGMT inhibitors or high dose alkylating agent therapy, increased toxicity is expected, because the normal tissue is not protected by endogenous levels of MGMT.

"BRCAness" and sensitivity to chemotherapy and PARP inhibitors. Mechanism-based strategies targeting loss of function of FA genes including BRCA2 comprise DNA cross-linking agents, whereas functional loss of BRCA1 also sensitizes to agents inducing double strand breaks. In accordance, the responsiveness

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4 http://clinicaltrials.gov, NCT00304031.
of patients to chemotherapy depending on the BRCA1 promoter methylation status is investigated in metastatic breast cancer with a regimen combining cisplatin and bevacizumab (NCT00517361). The sensitivity of "BRCAness" to inactivation of alternative repair pathways is evaluated with PARP inhibitors, as single agent or in combination with cisplatin, enrolling BRCA1-BRCA2-associated or hereditary breast and ovarian cancer patients (NCT00664781, NCT00647062). However, methylated BRCA1/2 is not considered as inclusion criteria in these trials.

Taking advantage of the epigenetically inactivated WRN gene. The reported high frequency of WRN promoter methylation in many tumor types makes it an attractive biomarker for stratified use of topoisomerase I inhibitors that are widely used as anticancer agents in many tumor types and potentially other DNA damaging drugs. The WRN promoter methylation status and benefit from therapy should be prospectively evaluated in ongoing studies to establish a predictive or a prognostic value.

Overcoming treatment resistance by restoring MMR using DNA demethylating agents. In contrast to epigenetic inactivation of the above-mentioned repair pathways, loss of MMR proficiency through promoter methylation of hMLH1 leads to resistance to chemotherapy. Thus, reactivation of MMR by restoring hMLH1
expression using DNA demethylating agents such as 5-aza-dC is an attractive approach. Azanucleoside drugs require incorporation into the DNA to inhibit DNMT-catalyzed DNA methylation. In accordance with this mechanism an ongoing trial for advanced ovarian, fallopian tube, and primary peritoneal cancer selects patients with methylated hMLH1 DNA in the plasma for treatment with 5-aza-dC in combination with cisplatin (NCT00748527). Recently, 5-aza-citidine and 5-aza-2′-deoxycytidine have been approved for myelodysplastic syndrome. In addition to a DNA-demethylating activity 5-aza-citidine also has a DNA-damaging effect inducing DNA repair signaling, which may account for part of its antitumor effect (44).

An alternative strategy may be to specifically target cancers in which the MMR repair pathway is perturbed. Compounds such as rhodium metalloinsertors bind to DNA base mismatches with high specificity and have been shown to inhibit cellular proliferation preferentially in MMR-deficient cells versus MMR-proficient cells (45).

**Conclusion**

As reviewed here, a number of tumor types exhibit clinically interesting frequencies of epigenetically inactivated DNA repair pathways that potentially render these tumors particularly sensitive to specific classes of therapeutic agents already in clinical use, or suggest novel avenues for drug development. Such stratified, personalized treatment strategies are supported by preclinical experiments. However, with the exception of MGMT methylation in glioblastoma, little is known about epigenetic inactivation of these repair pathways and their predictive value for benefit from therapies targeting their specific repair defects in larger cancer patient populations. Further, mechanistic correlations may be confounded by unknown associated alterations, e.g., a methylator phenotype, or conferring resistance such as an MMR defect in MGMT-methylated cells treated with alkylating agents. In addition, it is not always clear if inactivation of both alleles of an affected gene is required to elicit the desired effect. These new treatment opportunities for individualized therapy need to be translated into the clinical setting, which requires robust biomarker tests for systematic molecular profiling of the patients’ tumors for subsequent patient selection and evaluation in respective clinical trials.

**References**


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